SOME EFFECTS OF ACTH, CORTISONE, PROGESTERONE AND TESTOSTERONE ON SEBACEOUS GLANDS IN THE WHITE RAT*

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Sebaceous glands which are relatively small during childhood start to grow rapidly with the onset of puberty. At the same time there is a considerable increase in the amount of sebum excreted to the skin surface. This sebaceous gland hyper function is the cause of seborrhea in adolescents and is a major component of juvenile acne vulgaris.

The nature of the pubertal growth stimulus for sebaceous glands is not well understood, especially in females. That this stimulus does occur in females is evident from the approximately 1:1 sex incidence of acne (1). In males the testicular androgenic hormones have been implicated for the following reasons (2): 1. prepubertal castrate and eunuchoid males do not develop seborrhea or acne spontaneously; 2. they usually develop acne if they are treated with testosterone; and 3. normal males and females may develop acne if they are treated with large doses of testosterone. This hormone is particularly effective in producing acne when it is given to persons who have a genetic predisposition for this disease.

Animal experimentation has confirmed the clinical finding that male hormone enhances growth and secretion of sebaceous glands. De Graaf (3) found that sebaceous glands in the mid-dorsal region of rats were particularly sensitive to testosterone stimulation. Ebling (4) was the first to study this effect quantitatively. Hamilton and Montagna (5) administered androgen to hamsters and found increases in the size of sebaceous glands, individual cells, and secretory output within seventy-two hours. Decreases in size and number of rat sebaceous glands were noted when estrogen was given; also, "protection" from this effect was found when androgen was given concurrently (4, 6).

In girls it is easy to assume that increased androgens produced by the adrenals during puberty (7) are responsible for the development of sebaceous gland hyperfunction. It is, however, difficult to accept this view because male castrates and most ovarian deficient females have well functioning adrenal glands and yet do not develop acne or seborrhea.

Soon after the advent of therapy with adrenocorticotropin it was observed that many patients of all age groups, when treated with this hormone, developed acneiform eruptions. These acneiform eruptions, however, are not identical with juvenile acne because they lack the usual seborrhea and comedones (8). Clinically

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and histologically in these eruptions the major component is excessive follicular keratinization and there is little if any sebaceous gland hyperfunction. These observations, thus, also fail to support an adrenal cortical origin for sebaceous gland stimulation.

Local application of C-11 oxygenated steroids has resulted in inconsistent reduction in the size of rat sebaceous glands (9). Similar findings were noted upon parenteral administration of adrenocorticotropic hormone (10). One investigator has reported the failure of response of sebaceous glands to injections of progesterone (4).

The experiments to be reported here were designed to re-evaluate and extend the information available concerning the hormonal influences on sebaceous glands.

MATERIALS AND METHODS

Mature spayed female rats of the Sprague-Dawley strain were used. Each group of nine or ten rats was given daily subcutaneous injections of hormones for thirty days. The first group was given 0.5 mg. ACTH daily; the second group 1.0 mg. cortisone; the third group 1.0 microgram of progesterone; and a fourth control group received injections of peanut oil equivalent to the volume of progesterone solvent. In addition, a fifth group of animals was treated daily for only two weeks with 10 mgs. of progesterone. A sixth group was given 1.0 mgm. testosterone propionate daily for thirty days.

Skin biopsies were taken from the dorsal region of each rat before and after treatment. The biopsy specimens were pinned on cork boards, fixed in ten per cent formalin and prepared by routine paraffin technic. Thirty to forty serial sections, 10 micra in thickness, were cut from each block and stained with haematoxylin and eosin. Uniform handling of tissues was maintained throughout.

The sections were projected to provide one thousand fold magnification. Individual sebaceous gland alveoli were followed throughout their entirety in the serial sections. Tracings were made of the periphery of the glands as they appeared in consecutive sections, and the number of nuclei in each gland section was noted. Cross sectional areas were then determined by planimetry, permitting calculation of the approximate volume of individual glands as well as the cell count per unit volume. Six glands were chosen from each biopsy for volume determination and cell count. The only criteria employed in selection of glands were completeness within the set of serial sections and freedom from accidental trauma.

RESULTS AND DISCUSSION

The experimental data are summarized in the accompanying graph. Dotted lines indicate results in individual animals and the solid lines the averages. The results are expressed as percentage changes in volume following treatment. The greatest increase in sebaceous gland volume was observed in the animals treated with testosterone, namely an increase ranging from about 100 per cent to 900 per cent and averaging 400 per cent. However, the results with large dosage of progesterone come quite close to the effect of testosterone, namely a volume

increase of from about 100 per cent to 850 per cent with an average of 350 per cent. It was decided to use this large dose of progesterone after finding that as little as 1.0 microgram daily had a moderate but readily detectable effect on sebaceous gland volume. There was an increase in gland volume in all animals given this small dose and the average increase was about 80 per cent.

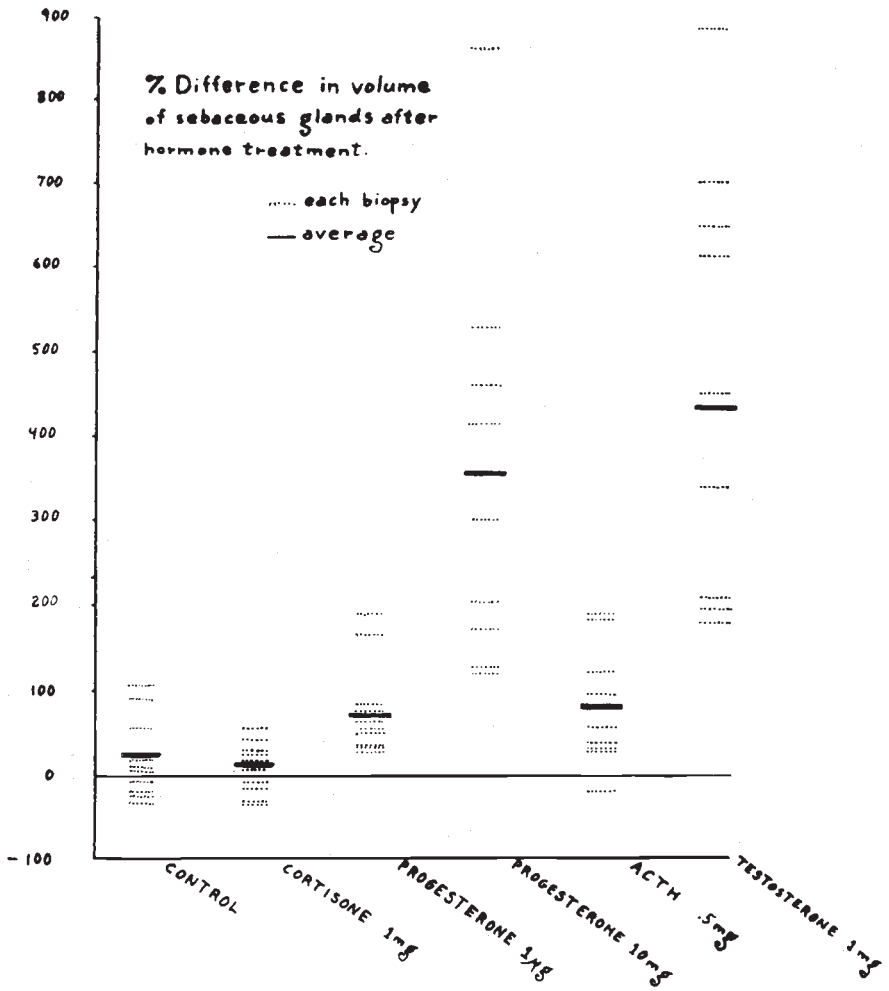

FIG. 1. Chart of experimental results

After treatment with both testosterone and progesterone, there was also a striking increase in the thickness of the basal layer of the glands and in the amount of free sebum at the gland orifices.

Cortisone treatment produced little if any significant variation in gland size as compared to pre-treatment biopsies. When compared with the control group there was slightly diminished volume. The cortisone dosage was obviously effec-

tive because all cortisone treated animals showed marked changes in and thinning of the dermis. Thus, the observation that the acneiform eruptions seen with cortisone treatment in humans lacks a seborrheic component has experimental support.

Results with ACTH were variable; most specimens showed enlargement but one showed diminution. Average increase for this group was 80 per cent. ACTH may have acted by stimulating adrenal androgen production.

The range of variation within each group may be accounted for in part by the physiological cyclic activity of sebaceous glands in the rat (11). Waves of glandular enlargement were found to alternate with the waves of increased hair growth reported by Butcher (12). The slight increment seen in the average value of the control group is in accord with Butcher's account of normal growth.

The ratio of nuclear count to alveolar volume proved to be nearly constant in all groups, showing that hormone-induced sebaceous gland enlargement is the result of hyperplasia and not simply hypertrophy, i.e. enlargement of single cells.

If the gland growth recorded in this paper is confirmed, a new hypothesis for the origin of adolescent seborrhea and acne in the female is suggested, i.e., that the initiation of ovulatory cycles and subsequent corpus luteum formation provides a source of seborrhea-producing progesterone. This hypothesis, since it does not implicate the adrenal, is consistent with the observation that hypogenital males and females do not develop seborrhea or acne.

SUMMARY

A series of quantitative experiments designed to estimate variations in size of rat sebaceous glands following injection of hormones is described.

Testosterone propionate (1.0 mg. daily) for thirty days produced an average increase in sebaceous gland size of about 400 per cent. Progesterone (10 mgs. daily) for fifteen days produced enlargement comparable to that induced by testosterone, i.e., an average increase of 360 per cent.

Cortisone (1.0 mg. daily) for thirty days caused mild sebaceous gland atrophy. ACTH (0.5 mg. daily) for the same time interval caused only slight volume increase which averaged about 80 per cent.

A new hypothesis concerning the endocrine origin of adolescent seborrhea and acne in the female is proposed.

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DISCUSSION

DR. MARION B. SULZBERGER, *New York, N. Y.:* This is one of the most interesting and important papers that it has been my privilege to hear for a long time and I want to congratulate the workers on a very illuminating and instructive piece of work well presented.

They said that males without testicles do not get acne and that females without testicles do not get acne. Formerly we blamed female acne on the adrenocortical hormones. But if one blames acne on those hormones in the female you could not explain why males with the same cortical hormones would not also get acne. The present authors have set up the theory that it is the progesterone which produces the female acne—therefore the female gets acne even though she has no testicle. They said another important thing: that it also depends upon how the target organ will respond to these circulating hormonal substances. I think that is brought out clearly in the acne which we find produced by cortisone or ACTH. It is our experience that young people with a tendency to acne are much more likely to get acne when cortisone or ACTH is administered; and that older women past the acne age are much more likely to get a beard when cortisone or ACTH is administered in sufficient dosages. It is the same hormones which produce both the acne and the hirsuties; but which of the two cutaneous responses is produced depends upon the age of the patient and the readiness of the particular pilo-sebaceous structure to respond. I think that indicates that there is something inherent in the organ of response which is important in determining the local reaction to the hormones, for example an "acne readiness."

DR. HERMANN PINKUS, *Detroit, Mich.:* I would like to ask Dr. Haskin if he made any observations concerning the stage of the hair cycle in those parts of the skin which he chose for examination. The hair of the rat grows in cycles, and growth waves which are accompanied by considerable variations in blood supply pass over the body of the animal periodically. Montagna and Chase (Anat. Rec. **107:** 83, 1950 and Proc. Soc. Exp. Biol. & Med. **76:** 35, 1951) have shown

that the responsiveness of the pilosebaceous complex to carcinogens and x-rays varies greatly depending on the stage of growth in which the individual hair happens to be. It seems possible that the magnitude of hormonal effects also may be influenced by the responsiveness of the hair follicle. If that is the case it would be necessary to compare the sebaceous glands of hair follicles in similar stages of growth.

DR. CLAYTON E. WHEELER, *Charlottesville, Virginia:* Some time ago, Dr. Curtis treated a group of patients with alopecia areata by giving them progesterone. I believe none of these patients developed acne, and I wish Dr. Curtis would say something about this.

DR. ARTHUR C. CURTIS, *Ann Arbor, Michigan:* These were long term studies on the use of progesterone in alopecia areata, but I do not remember any patients who developed acne on progesterone administration.

May I ask if the experimental animals used in this study were castrated, and was any other hormone, such as theelin, used?

DR. HERMAN CHASE, *Providence, R. I.:* A comparable change in the epidermis does occur during the normal hair cycle without treatment. It is very early in the hair cycle that you get this considerable increase in size and it lasts only two or three days, but in the normal you do get that very definite change.

DR. DAVID HASKIN, *Chicago, Ill. (in closing):* In answer to whether the changes in the size of sebaceous glands observed could have been physiological ones accompanying the hair cycle, I would like to point out that our values of absolute volumes in the testosterone-rats and progesterone-rats exceeded by several hundred per cent the average normal values and control values. This, of course, could not be caused by the hair cycle.

In answer to Dr. Curtis, the animals in these experiments were ovariectomized.

Concerning the effect of estrogens, in connection with Dr. Sulzberger's comments, we have a few rats that showed a definite decrease in sebaceous gland size from estrogen administration if there had been a preceding period of sebaceous gland stimulation. Our technic does not permit accurate assessment of decreases in gland size from normal because the normal size of the rat sebaceous gland is already so small that anything smaller can scarcely be measured.

DR. STEPHEN ROTHMAN, *Chicago, Ill. (in closing):* The point of Dr. Pinkus concerning hair cycle is well taken. We might have obtained more uniform results had we done our biopsies always in the same stage of the cycle. However, I cannot imagine that the uniformly powerful effect of testosterone and of progesterone and the uniform ineffectiveness of cortisone could have anything to do with different stages of the hair cycle.